REMARKS

The December 13, 2002 Official Action has been carefully reviewed. Applicants have incorporated proper sequence identifiers within the specification and claims as directed by the Examiner with the exception of page 35, lines 27-29. Applicants strenuously disagree with the Examiner's position that sequence identifiers are required within lines 27-29 of page 35. Indeed, careful inspection of these lines reveals no specifically defined nucleotide sequences of ten or more nucleotides or specifically defined amino acid sequences of four or more amino acids as required by 37 C.F.R. §1.821(a) to warrant a sequence identifier. Applicants contend the instant application, as amended, meets the requirements of 37 C.F.R. §§1.821-1.825.

Favorable consideration leading to prompt allowance of the present application is respectfully requested.

Respectfully submitted,

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By 

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Enclosure: -Marked up draft of amended specification and claims

Marked up draft of amended specification and claims

In the specification:

(Sheets 16 through 19 of the figures)

Table 2

mouse beta actin - 15mer peptides with 5 residue overlap:

1, MDDDIAALVVDNGSG = 1 - 15	(SEQ ID NO: 25)
2, AALVVDNGSGMCKAG = 6 - 20	(SEQ ID NO: 26)
3, DNGSGMCKAGFAGDD = 11 - 25	(SEQ ID NO: 27)
4, MCKAGFAGDDAPRAV = 16 - 30	(SEQ ID NO: 28)
5, FAGDDAPRAVFPSIV = 21 - 35	(SEQ ID NO: 29)
6, APRAVFPSIVGRPRH = 26 - 40	(SEQ ID NO: 30)
7, FPSIVGRPRHQGVMV = 31 - 45	(SEQ ID NO: 31)
8, GRPRHQGVMVGMGQK = 36 - 50	(SEQ ID NO: 32)
9, QGVMVGMGQKDSYVG = 41 - 55	(SEQ ID NO: 33)
10,GMGQKDSYVGDEAQS = 46 - 60	(SEQ ID NO: 34)
11,DSYVGDEAQSKRGIL = 51 - 65	(SEQ ID NO: 35)
12,DEAQSKRGILTLKYP = 56 - 70	(SEQ ID NO: 36)
13,KRGILTLKYPIEHGI = 61 - 75	(SEQ ID NO: 37)
14,TLKYPIEHGIVTNWD = 66 - 80	(SEQ ID NO: 38)
15,IEHGIVTNWDDMEKI = 71 - 85	(SEQ ID NO: 39)
16,VTNWDDMEKIWHHTF = 76 - 90	(SEQ ID NO: 40)
17,DMEKIWHHTFYNELR = 81 - 95	(SEQ ID NO: 41)
18,WHHTFYNELRVAPEE = 86 - 100	(SEQ ID NO: 42)
19,YNELRVAPEEHPVLL = 91 - 105	(SEQ ID NO: 43)
20,VAPEEHPVLLTEAPL = 96 - 110	(SEQ ID NO: 44)
21,HPVLLTEAPLNPKAN = 101 - 115	(SEQ ID NO: 45)
22,TEAPLNPKANREKMT = 106 - 120	(SEQ ID NO: 46)
23,NPKANREKMTQIMFE = 111 - 125	(SEQ ID NO: 47)
24,REKMTQIMFETFNTP = 116 - 130	(SEQ ID NO: 48)
25,QIMFETFNTPAMYVA = 121 - 135	(SEQ ID NO: 49)
26,TFNTPAMYVAIQAVL = 126 - 140	(SEQ ID NO: 50)
27,AMYVAIQAVLSLYAS = 131 - 145	(SEQ ID NO: 51)

28,IQAVLSLYASGRTTG = 136 - 150 (SEQ ID NO: 52)
29,SLYASGRTTGIVMDS = 141 - 155 (SEQ ID NO: 53)
30,GRTTGIVMDSGDGVT = 146 - 160 (SEQ ID NO: 54)
31,IVMDSGDGVTHTVPI = 151 - 165 (SEQ ID NO: 55)
32,GDGVTHTVPIYEGYA = 156 - 170 (SEQ ID NO: 56)
33,HTVPIYEGYALPHAI = 161 - 175 (SEQ ID NO: 57)
34,YEGYALPHAILRLDL = 166 - 180 (SEQ ID NO: 58)
35,LPHAILRLDLAGRDL = 171 - 185 (SEQ ID NO: 59)
36,LRLDLAGRDLTDYLM = 176 - 190 (SEQ ID NO: 60)
37,AGRDLTDYLMKILTE = 181 - 195 (SEQ ID NO: 61)
38,TDYLMKILTERGYSF = 186 - 200 (SEQ ID NO: 62)
39,KILTERGYSFTTTAE = 191 - 205 (SEQ ID NO: 63)
40,RGYSFTTTAEREIVR = 196 - 210 (SEQ ID NO: 64)
41,TTTAEREIVRDIKEK = 201 - 215 (SEQ ID NO: 65)
42,REIVRDIKEKLCYVA = 206 - 220 (SEQ ID NO: 66)
43,DIKEKLCYVALDFEQ = 211 - 225 (SEQ ID NO: 67)
44,LCYVALDFEQEMATA = 216 - 230 (SEQ ID NO: 68)
45,LDFEQEMATAASSSS = 221 - 235 (SEQ ID NO: 69)
46,EMATAASSSSLEKSY = 226 - 240 (SEQ ID NO: 70)
47,ASSSSLEKSYELPDG = 231 - 245 (SEQ ID NO: 71)
48,LEKSYELPDGQVITI = 236 - 250 (SEQ ID NO: 72)
49,ELPDGQVITIGNERF = 241 - 255 (SEQ ID NO: 73)
50,QVITIGNERFRCPEA = 246 - 260 (SEQ ID NO: 74)
51,GNERFRCPEALFQPS = 251 - 265 (SEQ ID NO: 75)
52,RCPEALFQPSFLGME = 256 - 270 (SEQ ID NO: 76)
53,LFQPSFLGMESCGIH = 261 - 275 (SEQ ID NO: 77)
54,FLGMESCGIHETTFN = 266 - 280 (SEQ ID NO: 78)
55,SCGIHETTFNSIMKC = 271 - 285 (SEQ ID NO: 79)
56,ETTFNSIMKCDVDIR = 276 - 290 (SEQ ID NO: 80)
57,SIMKCDVDIRKDLYA = 281 - 295 (SEQ ID NO: 81)
58,DVDIRKDLYANTVLS = 286 - 300 (SEQ ID NO: 82)
59,KDLYANTVLSGGTTM = 291 - 305 (SEQ ID NO: 83)
60,NTVLSGGTTMYPGIA = 296 - 310 (SEQ ID NO: 84)
61,GGTTMYPGIADRMQK = 301 - 315 (SEQ ID NO: 85)
62,YPGIADRMQKEITAL = 306 - 320 (SEQ ID NO: 86)

63,DRMQKEITALAPSTM = 311 - 325 (SEQ ID NO: 87)
64,EITALAPSTMKIKII = 316 - 330 (SEQ ID NO: 88)
65,APSTMKIKIIAPPER = 321 - 335 (SEQ ID NO: 89)
66,KIKIIAPPERKYSVW = 326 - 340 (SEQ ID NO: 90)
67,APPERKYSVWIGGSI = 331 - 345 (SEQ ID NO: 91)
68,KYSVWIGGSILASLS = 336 - 350 (SEQ ID NO: 92)
69,IGGSILASLSTFQQM = 341 - 355 (SEQ ID NO: 93)
70,LASLSTFQQMWISKQ = 346 - 360 (SEQ ID NO: 94)
71,TFQQMWISKQEYDES = 351 - 365 (SEQ ID NO: 95)
72,WISKQEYDESGPSIV = 356 - 370 (SEQ ID NO: 96)
73,EYDESGPSIVHRKCF = 361 - 375 (SEQ ID NO: 97)
74,GGGGGGPSIVHRKCF = 366 - 375 (SEQ ID NO: 98)
75,GGGGGGGGGGHRKCF = 371 - 375 (SEQ ID NO: 99)

Other peptides to include:

76,KYSVWIGGSILASLS (SEQ ID NO: 100)
alpha helix in subdomain 1 of rabbit alpha actin- contains two hydrophobic residues accessible to solvent
(residues S338 - S348)

77,PRHQGVMVGMGQKDS (SEQ ID NO: 101)
loop in subdomain 2 of rabbit alpha actin- major interaction site with DNase I
(residues P38 - S52)

78,IVLDSGDGVTHNVPI (SEQ ID NO: 102)
beta strands in subdomain 3 of rabbit alpha actin
(residues G150 - Y166)

79,LVCDNGSGLVKAGFA (SEQ ID NO: 103)
analagous beta strand motif in subdomain 1 of rabbit alpha actin
(residues L8 - F21)

80,LFQPSFIGMESAGIH (SEQ ID NO: 104)
loop in subdomain 4 of rabbit alpha actin- involved in contact across helix axis in F-actin
(residues F262 - I274)

81,TTAEREIVRDIKEKL (SEQ ID NO: 105)
alpha helix in subdomain 4 of rabbit alpha actin- minor interaction site with DNase I
(residues T203 - L216)

82,YVGDEAQSKRGILTL (SEQ ID NO: 106)
beta alpha beta unit in subdomain 2 of rabbit alpha actin- minor interaction site with DNase I/ hexokinase-like unit
(residues K61 - L65)

83,VMSGGTTMYPGIADR (SEQ ID NO: 107)
loop in subdomain 3 of rabbit alpha actin- forms pocket for adenine base of nucleotide
(residues S300 - I309)

84,KIKIIAPPERKYSVW (SEQ ID NO: 108)
beta strand and loop in subdomain 3 of rabbit alpha actin- forms pocket for adenine base of nucleotide
(residues K328 - S338)

85,GFAGDDAPRAVFPSI (SEQ ID NO: 109)
loop in subdomain 1 of rabbit alpha actin- central contact region of myosin on 'flat' side of actin
(residues F21 - P32)

86,YNELRVAPEEHPTLL (SEQ ID NO: 110)
loop in subdomain 1 of rabbit alpha actin- contact region of myosin on 'flat' side of actin
(residues N92 - T103)

87,TFQQMWITKQEYDEA (SEQ ID NO: 111)
alpha helices in subdomain 1 of rabbit alpha actin- bind myosin chains
(residues S348 - A365)

88,DEDETTALVCDNGSG (SEQ ID NO: 112)
N-terminal 15 residues of rabbit alpha actin- important in binding myosin
(residues D1 - G15)

89,EYDEAGPSIVHRKCF (SEQ ID NO: 113)
C-terminal 15 residues of rabbit alpha actin
(residues E361 - F375)

90,SKQEYDESGPSIVHR (SEQ ID NO: 114)
truncated C-terminus of mouse beta actin
(residues S358 - R372)

91,ILTERGYSFVTTAER (SEQ ID NO: 115)
loop in subdomain 4 of rabbit alpha actin- analagous to DNase I-binding loop in subdomain 2
(residues T194 - T203)

92,ALDFENEMATAASSS (SEQ ID NO: 116)
alpha helix flanked by loops in subdomain 4 of rabbit alpha actin
(residues F223 - A230)

93,WDDMEKIWHHTFYNE (SEQ ID NO: 117)
alpha helix in subdomain 1 of rabbit alpha actin
(residues W79 - N92)

94,+ve control for 91a = STDLVAKLRAFHNEA (SEQ ID NO: 118)

(Page 33, line 9) **Figure 8** shows newly synthesized

CCT subunits are incorporated into CCT semi-conservatively. (A) The protein sequences of the C-termini of rabbit (SEQ ID NO: 16), wildtype (SEQ ID NO: 16) and mutant mouse CCTα (SEQ ID NO: 17). (B) The difference in CCT migration distance induced by monoclonal antibody 23C after incorporating either wildtype mouse CCTα (lane 2) or mutant CCTα (lane 4) is clearly discernable. Lane 1 and 3 represents the migration of CCT without exposure to antibody 23C after incorporating either wildtype mouse CCTα (lane 1) and mutant mouse CCTα (lane 3). (C) A pictorial representation of the coupling of two antibody molecules onto rabbit endogenous CCT and one antibody molecule coupled onto CCT containing an incorporated mutant mouse CCTα subunit (subunit in black).

(Page 33, line 28) **Figure 10** shows the peptide sequences refered to in Figure 11. The Reference Peptide Nos are the SEQ ID NOs of the listed peptide sequences.

(Page 33, line 31) **Figure 11** shows the interaction of actin derived peptides and alanine scanning mutations of actin derived peptides with CCT. Mouse testis CCT was incubated singly or in combination with [the illustrated peptides (Figure 11A)] peptide 8 (lanes 1 and 2; SEQ ID NO: 18), peptide 8.1 (lane 3; SEQ ID NO: 19), peptide 8.2 (lane 4, SEQ ID NO: 20), peptide 8.3 (lane 5; SEQ ID NO: 21), peptide 8.4 (lane 6; SEQ ID NO: 22), peptide 8.5 (lane 7; SEQ ID NO: 23), or peptide 8.6 (lane 8; SEQ ID NO: 24) as listed in Figures 11A and 11C. In all lanes, CCT was incubated with peptide on ice for one hour. Samples were electrophoresed on 6% native gels, transferred to nitrocellulose membrane and incubated with Neutravidin-HRP (Pierce) at 2 μg per ml to reveal the distribution of biotinylated peptides. The arrowed region (Figure 11B) shows CCT complexes bound by peptides. The sequences in Figure 11B (lane 3, residues 5 to 9 of SEQ ID NO: 19; lane 4, residues 5 to 9 of SEQ ID NO: 20; lane 5, residues

5 to 9 of SEQ ID NO: 21; lane 6, residues 5 to 9 of SEQ ID NO: 22; lane 7, residues 5 to 9 of SEQ ID NO: 23; lane 8, SEQ ID NO: 122) are the core sequences of the mutant Actin Site I sequences present in the peptides listed in Figures 11A and 11C. Figure 11C shows the results quantitated.

(Page 51, line 21) The present inventors have found that substitution of the sequence GRPRH (SEQ ID NO: 121) by sequential alanine residues within peptide 8 significantly depletes or enhances binding to CCT in this free solution assay (Fig. 11c). This further confirms the ability of the methodologies embodied herein to identify protein-protein interaction sites, to find the minimal number of residues responsible for binding within a BEP and to perform mutation analysis on the BEPs to modify the efficacy of BEP binding.

(Page 56, line 13) The present inventors have focused their attention on β-Actin Site I, a high-affinity site which occupies three overlapping peptides and spans amino acid residues 26-50 of actin subdomain 2. They demonstrated the interaction between CCT and N-terminally biotinylated peptide in solution. CCT and peptide corresponding to β-Actin Site I (Fig. 10, peptide 8) were incubated together and the reactions were then electrophoresed on native PAGE gels, western blotted and probed with streptavidin-Horse Radish Peroxidase (HRP). A biotin signal co-migrating with CCT was detectable within a 10-fold concentration range of peptide (1.33 μM to 13.3 μM) and fixed concentration of CCT (70nM). Five alanine-scan point mutations across the core sequence (^{36}GRPRH^{40}; SEQ ID NO: 121) of β-Actin Site I were screened for effects on interaction with CCT. The mutant peptides showed equivalent, reduced or enhanced binding, but not absence of binding, although replacement of all five residues of the GRPRH (SEQ ID NO: 121) core sequence by AAAAA (SEQ ID NO: 122) resulted in abrogation of binding to CCT. The inventors noted

that the *act1-132* mutant allele of the yeast actin gene, *ACT1*, which contains a double alanine replacement in the core, ^{36}GAPAH^{40} (SEQ ID NO: 123), has a recessive Cs$^-$, Ts$^-$ phenotype *in vivo* (Wertman et al, Genetics 132, 337-350, 1992).

(Page 63, line 7) A set of seventy-three Pepset™ peptides (Meltek Scientific Ltd) scanning the 375 amino acid residues of mouse β-actin sequence were synthesized on polyethylene solid phase pins in a 96-well format. Each peptide was 15 residues in length; starting from the amino terminal peptide, (#1) ^{1}MDDDIAALVVDNGSG^{15} (SEQ ID NO: 25) each subsequent peptide was offset by 5 residues, i.e. (#2) ^{6}AALVVDNGSGMCKAG^{20} (SEQ ID NO: 26), (#3[0]) ^{11}DNGSGMCKAGFAGDD^{25} (SEQ ID NO: 27) etc. To detect the interaction of holochaperonin or isolated chaperonin apical domains with the immobilised peptide array, an assay was developed involving monoclonal antibody (MAb) binding followed by ELISA detection. Non-specific binding to the peptide pins was reduced by incubation with pre-coat buffer (2% BSA, 0.1% Tween 20 in PBS.A pH7.2) for one hour at room temperature. Chaperonin (CCT, GroEL or isolated CCT5 apical domain) was diluted to a concentration of X-Yμg/ml in binding buffer (50mM HEPES pH 7.2, 90mM KC1, 0.5mM $MgCl_2$) and incubated with the peptide pins for 16 hours at 4°C. The pins were washed three times with PBS for a total of 30 minutes, and incubated with the appropriate MAb for 2 hours at room temperature; CCT was detected using MAb 91a (Willison et al Cell, 57, 621-632, 1989), which recognizes the CCTα subunit, GroEL was detected by MAb 4-3F (a kind gift from Dr P Lund, University of Birmingham) and isolated CCTδ apical domain, tagged with a C-terminally located -GALDD (SEQ ID NO: 119) pentapeptide, was detected by MAb 23c (Harrison Lavoie, EMBO J. 12, 2847-2853, 1993) or one isolated CCTδ apical domain, tagged at the C-terminus with a His6 motif, was detected by MAb HIS1 (Sigma). Following washing in PBS, the pins were incubated with a secondary

antibody conjugated to alkaline phosphatase (5μg/ml in PBS, Pierce) for 2 hours at room temperature. The pins were washed in PBS and incubated with p-Nitrophenyl phosphate (Sigma) in a 96-well microtitre plate for 30 minutes in the dark. Absorbance at 410nm due to the conjugates was detected using an ELISA plate reader.

(Page 64, line 18) A set of seventy-four PepsetTM peptides (Meltek Scientific) was synthesized on polyethylene solid phase pins in a 96-well format. Each peptide was immobilised at the C- terminus and contained 15 amino acid residues and an acid N-terminus. Peptides 1 to 73 scanned the primary structure of mouse cytoplasmic β-actin (SwissProt:P02570), and starting from the amino terminal peptide (#1) ^{1}MDDDIAALVVDNGSG^{15} (SEQ ID NO: 25) each subsequent peptide was offset by 5 residues, i.e. (#2) ^{6}AALVVDNGSGMCKAG^{20} (SEQ ID NO: 26), (#3) ^{11}DNGSGMCKAGFAGDD^{25} (SEQ ID NO: 27) etc, Peptide 74 contained the epitope sequence for monoclonal antibody (MAb) 91a, which recognizes CCTα (Willison et al, Cell, 57, 621-632, 1989). An assay to detect the interaction of molecular chaperone proteins with the peptide array involved MAb binding followed by ELISA detection. Non-specific binding to the peptide pins was reduced by incubation with pre-coat buffer (2% BSA, 0.1% Tween 20 in PBS.A pH7.2) for one hour at room temperature. Purified molecular chaperones (6.5μg/ml) or 6.5nM CCT; 1.25UG/ML] or 1.47nM GroEL; 4 fractions (2ml total) of purified CCTδ apical domain to a volume of 0.2 ml and the protein concentration was calculated to be 0.6 mg/ml approximately 3.6μg/ml isolated CCTδ apical domain; or 0.675μg.ml or 6.75μM Hsp70) in binding buffer (50mM HEPES pH 7.2, 90mM KC1, 0.5mM $MgCl_2$) were incubated with the peptide array for 16 hours at 4°C. The pins were washed three times with PBS for a total of 30 minutes and incubated with the appropriate MAb (approximately 1.5μg/ml in PBS) for 2 hours at room temperature; CCT was detected with MAb 91a

(Willison et al Cell, 57, 621-632, 1989), GroEL was detected with MAb 4-3F (a kind gift from Dr P Lund, University of Birmingham), Hsp70 was detected with Mab 3A3(Affinity Bioreagents), and isolated CCTδ_apical domain tagged at the C-terminus with a -GALDD (SEQ ID NO: 119) pentapeptide was detected with MAb 23c (Willison et al Cell, 57, 621- 632, 1989) . Following washing in PBS, the pins were incubated with a secondary antibody conjugated to alkaline phosphatase (30ng/ml in PBS, Pierce) for 2 hours at room temperature. The pins were washed in PBS and incubated with p-Nitrophenyl phosphate (Sigma) in 96-well microtitre plate for 30 minutes in the dark. Absorbance at 410nm due to the conjugates was detected using a microplate reader (Model MR 710, Dynatech).

(Page 66, line 1) Biotinylated Pepset™ peptides (Meltek Scientific) corresponding to residues 36-50 of mouse β-actin were synthesized on polyethylene solid phase pins, and were chemically cleaved from the solid support to release the peptides. Each peptide contained an amide C-terminus and 19 amino acid residues including a -SGSG (SEQ ID NO: 120) linker to a biotin group at the N-terminus. The set consisted of the wild- type β-actin sequence (biotin-SGSG-^{36}GRPRHQGVMVGMGQK^{50}, SEQ ID NO: 18), five mutant peptides containing alanine scanning substitutions of residues GRPRH (SEQ ID NO: 121) (biotin-SGSG-ARPRHQGVMVGMGQK, SEQ ID NO: 19;
biotin-SGSG-GAPRHQGVMVGMGQK, SEQ ID NO: 20;
biotin-SGSG-GRARHQGVMVGMGQK, SEQ ID NO: 21;
biotin-SGSG-GRPAHQGVMVGMGQK, SEQ ID NO: 22; and
biotin-SGSG-GRPRAQGVMVGMGQK, SEQ ID NO: 23)
and one mutant peptide where all five residues of the GRPRH (SEQ ID NO: 121) core sequence were replaced by AAAAA (SEQ ID NO: 122)(biotin-SGSG- AAAAAQGVMVGMGQK, SEQ ID NO: 24). The peptides were solubilised in 10% acetic acid and analysed by MALDI-MS on a Finnegan Lasermat 2000, and peptide concentration was determined by amino acid analysis. CCT

(70nM) was incubated with peptide (13.3μm or 1.33μM) in binding buffer (50mM HEPES pH 7.2, 90mM KCl, 0.5mM $MgCl_2$) for one hour on ice. CCT complex was resolved on 6% native-PAGE gels, electrotransferred to ntirocellulose membrane, incubated with Neutravidin-HRP (Pierce) (2ug/ml in 2% BSA/PBS) in order to detect the interaction between CCT and biotinylated peptide.

In the claims:

10. (Twice Amended) A method according to claim 9 wherein the binding member comprises the amino acid sequence GRPRH (SEQ ID NO: 121).

24. (Amended) A binding member according to claim 23 comprising the amino acid sequence GRPRH (SEQ ID NO: 121).